Causes of agronomic differences between synthetics developed by the random and convergent cross methods

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Abstract. The theoretical utility of two alternative methods, random (r) and convergent (c) cross methods, of producing maize synthetics has previously been ascertained in two different genetic backgrounds (EPS20 derived from eight Reid inbred lines with origin from the U.S.Corn Belt population "Reid", and EPS21 formed from eight non-Reid inbreds). However, the agronomical consequences of using one or another methodology have not been tested. The objectives of the present study were to determine, in two genetic backgrounds, whether synthetics developed by the random and convergent cross methods differed in agronomic performance and to investigate whether some allelic changes previously observed by Butron et al (2003) could be directly implicated in those differences. The synthetics and the diallel crosses among them, testcrosses of EPS20c and EPS20r to their Reid parental inbreds and testcrosses of EPS21c and EPS21r to their non-Reid parental inbreds were evaluated for grain yield in three trials in 2004 and 2005. Our results suggest that directional selection for germination, which occurs during the process of formation of synthetics using the random method (but absent with the convergent cross method) was responsible for agronomic and genetic differences between synthetics obtained by alternative methods from the same set of inbreds. Although selection for germination increased the yield performance of the synthetic obtained from the Reid inbreds, in a more heterogeneous genetic background, natural selection against non-competitive inbred lines at germination would be responsible for an important reduction of variability that would reduce yield.

The term synthetic variety has been used extensively to refer to those populations that result after randomly mating a balanced bulk of several inbred lines or populations. According to this broad definition, synthetic varieties are produced by mating several parents so that all possible crosses between the parents have equal probability of being represented in the synthetic. Mating randomly all possible (diallel) crosses among *n* lines has been proposed as the most effective procedure for developing synthetic varieties (Allard 1960). When the number of lines is large, however, this protocol becomes too burdensome, and a labor and time saving method is desirable.

Maintaining the original genetic diversity in a synthetic can have practical implications because, according to Busbice (1970), yield of a synthetic (Y_{Syn}) depends on yield of homozygotes (Y_{Hom}) in the ancestry of the population plus the product of the yield of the heterozygote (Y_{Het}) multiplied by the difference between one and the inbreeding coefficient (F):

$$Y_{Syn} = Y_{Hom} + Y_{Het} (1 - F)$$

Thus, loss of diversity due to factors such as genetic drift can result in reduced yield of the synthetic. Variability leaks should also be avoided in the process of producing synthetics when the final goal of the population is the development of inbred lines, in order to prevent losses of potentially useful alleles.

Márquez-Sánchez (1992; 1993) found that the inbreeding coefficients of synthetic varieties obtained by different mating methods were the same regardless of whether the first generation is obtained by intercrossing lines or by randomly mating the plants from a seed bulk. He established that any random mating method could be useful as long as each component line had the same chance of contributing to the synthetic variety. However, factors leading to non-balanced contribution of inbreds to the synthetic can result in loss of genetic variability.

The two main factors that can change allele frequencies of a synthetic developed from a random sample of plants from a parental seed bulk are as follow: i) natural selection triggered by heritable differences among lines in germination success, vigor, seed production, etc. For example, Revilla et al. (2000) showed that reduced germination of *su1* kernels could account for most of the decrease in *su1* frequency in crosses between *su1* and *Su1* maize populations across generations of recombination. Under these circumstances, the expected number of parents involved in any sample drawn from the bulk will be less than the number of parents included in the bulk (Crossa 1989), (ii) genetic drift associated with reduced effective population size that can cause random fluctuations in allele frequencies (Crossa 1989). However, when synthetics are the base material for breeding programs, those genetic changes could be of little importance if no impact is observed on the agronomical performance of synthetics. In summary, when considering alternative mating methods for the development of synthetic varieties, not only should the methods be simpler and faster, but they should also prevent the unequal contribution of parents and random genetic drift (if conservation of genetic variability is the goal) and maximize agronomic performance (if selection is intended).

In a previous study, the theoretical utility of two alternative methods of producing maize synthetics was ascertained in two different genetic backgrounds (eight Reid inbred lines originated from the U.S.Corn Belt population "Reid", and eight non-Reid inbreds), assuming an equal contribution of each parental inbred line (Butrón et al. 2003). Results showed that the convergent cross method could modify gene frequencies of some SSR markers if few individuals were sampled among segregating-individuals of each double cross hybrid, but could not cause allele losses or significant reduction of heterozygosity (Butrón et al. 2003). On the other hand, the random method caused drastic deviations of allelic frequencies from expected ratios in a synthetic developed from non-related materials,

decreasing significantly the heterozygosity, and modifying genetic distances between the synthetic and their parental inbreds. Based on those results, the convergent cross method was found to be a valid method in both backgrounds. It was concluded that the random method should be used with caution when the inbreds intermated are genetically diverse. However, to determine the real utility and the risk associated with the use of the random method, the agronomical performance of a synthetic developed by the random method should be compared to that of the synthetic developed by the cross convergent method. The objectives of the present study were to determine, in two genetic backgrounds, whether synthetics developed by the random and convergent cross methods differed in agronomic performance and to investigate, through data mining, whether some allelic changes previously observed by Butron et al (2003) could be directly implicated in those differences.

Materials and methods

The synthetic varieties EPS20 and EPS21 were developed using two mating methods, referred to as convergent cross (EPS20c and EPS21c) and random (EPS20r and EPS21r) methods. Eight Reid inbred lines originated from the US Corn Belt population "Reid" and eight inbreds that were unrelated to "Reid" population were the base materials for synthetic varieties EPS20 and EPS21, respectively (Table 1). The synthetics EPS20c and EPS20r were formed from inbreds lines derived from B14 or WF9, both of which originated, directly or indirectly, from the population "Reid" (Messmer et al. 1991; Gerdes et al. 1993). The synthetics developed by the convergent cross method (c) were obtained from specific crosses involving *n* parental inbreds. Specifically, the 8 parents were crossed in pairs, then the 4 hybrids were crossed to make 2 double-crosses, and so on until a final cross involving all *n* parents was completed. The random method (r) involved random intermating of a sample of plants obtained by bulking equal number of seeds from each parental line.

In the convergent cross method, the single crosses, CM109 × CM151, A652 × A664, W64A × A634, and A639 × CM139, were made in 1995 as the first step in forming the balanced synthetic variety EPS20c. Crosses A509 × CO125, PB60 × PB130, F473 × EP53, and EP17 × EP43 were also made the same year to form the balanced synthetic variety EPS21c. The double crosses, (CM109 × CM151) × (A652 × A664) and (A639 × CM139) × (A634 × W64A) were produced for EPS20c, and (A509 × CO125) × (PB60 × PB130), and (F473 × EP53) × (EP17 × EP43) for EPS21c in 1996. Finally, in 1997, crosses between the two double cross-hybrids within each synthetic variety were made to obtain the synthetic varieties EPS20c and EPS21c. For each synthetic, equal number of seeds from each ear was bulked and three hundred seeds out of the bulk were sown in ten rows

each with 15 hills and two seeds per hill. The seedlings were later thinned to one plant per hill leaving 150 plants for plant-to plant crosses. Each plant was used only once as male or female resulting in at least 50 ears. Equal numbers of seeds were bulked from each ear and another generation of recombination was carried out.

To initiate the random method, three hundred and four seeds from the eight inbred lines that constituted the base material for each synthetic variety (EPS20r and EPS21r) were bulked and sown in 1998. Each inbred line contributed 38 seeds to the bulk. The 304 seeds were sown in ten rows, each with 15 hills and two seeds per hill. Following thinning, 150 plants were available to form each random synthetic variety (EPS20r and EPS21r). Plant-to plant crosses were made using each plant only once as male or female. This resulted in 38 and 39 ears that constituted the synthetic varieties, EPS20r and EPS21r, respectively, after two generations of recombination. Recombinations were made as described earlier for synthetics EPS20c and EPS21c.

In 2002, the promising diallel crosses of the four maize synthetic populations (EPS20c, EPS20r, EPS21c, and EPS21r) were made, and the synthetics were multiplied to obtain homogeneous seed. More than 50 ears were obtained for each cross and synthetic. Furthermore, each synthetic was crossed to each of its parental inbreds in 2002 and 2003 using the synthetics as males. Bulk pollen from a minimum of 50 male plants of each synthetic was used to pollinate more than 30 females of each inbred. The crosses A639 × EPS20r and EP43 × EPS21c failed in both years and were therefore not included in field evaluations.

The diallel crosses and parental populations, testcrosses of EPS20c and EPS20r to Reid parental inbreds and testcrosses of EPS21c and EPS21r to non-Reid parental inbreds were evaluated in three adjacent trials in 2004 and 2005, at Pontevedra. For each trial, a randomized complete block design with 3 replications was used. Each genotype was

planted in a two-row plot with 17 hills per row. The rows were spaced 0.80 m with 0.21 m between hills. Two seeds were planted per hill and later thinned to one resulting in a final population density of about 60 000 plants ha^{-1}.

Grain yield, the most important agronomic trait, has important dominance genetic effects. Therefore, it is expected that crosses between genetically distinct varieties would produce larger yields than genetically related varieties. Grain yield was computed as the shelled grain weight at 140 g kg^{-1} moisture per plot converted to Mg ha^{-1}.

Combined analyses of variance across years were performed on diallel data including parental populations using the PROC GLM procedure of SAS (SAS Institute 2002). Genotypes were considered fixed, and years and replications random. Midparent heterosis was estimated as the mean of crosses minus the mean of parental populations. The standard error of heterosis was calculated as the square root of 1.5 times the variance of the entry mean, according to the method of Keeratinijakal and Lamkey (1993). All analyses were made using SAS, version 9.1 (SAS Institute 2002). Combined analyses of variance across years were also performed, independently for each background (Reid and non-Reid) synthetic, on testcrosses data of synthetics to their parental inbreds. Testcrosses were assumed to be fixed effects. Mean comparisons among the genotypes of the diallel design and among testcrosses of synthetics to their parental inbreds were made using the Fisher's protected LSD.

In a previous study (Butrón et al. 2003), 40 individuals from each synthetic (EPS20c, EPS20r, EPS21c, and EPS21r) and parental inbreds were genotyped with several polymorphic SSRs (*phi083, nc132, phi090, phi036, bnlg197, phi046, phi021, phi076, phi113, phi101, phi128, phi075, phi112, phi114, phi116, phi115, bnlg240, phi028,phi065, phi027,* and *phi050*) randomly distributed across the maize genome, except on chromosome 1 . Marker locations and primer sequences could be down loaded from the Maize Genetics and

Genomics Database (http://www.maizegdb.org). In the present study, we have investigated, through data mining in the 'Maize Genetics and Genomics Database' (MaizeGDB), accessible through http://www.maizegdb.org, whether each of the 12 loci detected by Butrón et al. (2003) as showing allelic frequencies significantly different from expected (under the assumption of equal contribution of each inbred) are located on known genes that could be directly implicated in agronomic differences between synthtetics. Besides, genetic distances among synthetics varieties developed from the same materials by different methods were computed according to Nei (1972) using the program NTSYS-PC (Rohlf, 1997) and empirical estimates for the effective population size were obtained as well as their 95 % confidence intervals using temporal method of Waples (1989). Expected allele frequencies under the assumption of equal contribution of parental lines to the synthetic variety were assumed as the frequencies of an ideal initial sample of 10^{10} individuals (generation 1), while the variety synthetic was the generation 3. The standardized variance in allele frequency change (F_c) was calculated following the method of Nei and Tajima (1981) for all loci and for loci that exhibited allelic frequencies not significantly different from the expected ones (neutral loci). To avoid the possible bias in the estimation of the effective population size caused by alleles at initially high frequencies, loci with an expected frequency of the most common allele larger than 0.90 were removed (Labate et al. 1999).

Results

Nei's genetic distance between EPS20c and EPS20r was 4.2, while the distance between EPS21c and EPS21r was almost double, 7.9. EPS20c and EPS21c did not differ in grain yield, but EPS20r yielded significantly more than EPS21r (Table 2). In the diallel analysis,

the genotypes that showed the highest grain yield were EPS20r × EPS21c and EPS20c × EPS21c. On the other hand, EPS20c × EPS21r and EPS20r × EPS21r were similar to the synthetics *per s*e in grain yield, except EPS21r, and the crosses between synthetics developed by alternative methods from the same set of inbred lines (EPS20c × EPS20r and EPS21c × EPS21r). Crosses EPS20c × EPS21c and EPS20r × EPS21c showed significant heterosis for grain yield.

The analysis of variance of testcrosses of EPS20c or EPS21c to their parental inbreds did not show any significant differences for grain yield. However, significant differences were detected in grain yield of crosses of EPS20r or EPS21r with their parental inbreds (Table 3). The crosses CM151 × EPS20r, A634 × EPS20r, A652 × EPS20r, and W64A × EPS20r were among the highest yielding genotypes while CM139 × EPS20r was the least productive. EPS21r testcrossed to EP17, EP53, CO125, and A509 yielded significantly less than the best testcross, F473 × EPS21r (Table 3).

The estimated effective numbers computed from all loci were similar to the expected ones for the variety synthetics developed by the convergent cross method, but the estimated effective numbers were significantly lower than expected for synthetics obtained by the random method (Table 4). The estimated effective numbers computed with neutral loci approximated the expected effective numbers in all cases; while when computed with non neutral loci only they approximated the expected ratios in EPS21c.

The SSR markers for which Butrón et al. (2003) detected allelic frequencies significantly different from expected are shown in Table 5, along with information on the inbred lines that reduced or increased their contributions to the synthetic, and the locus where the SSR marker is located. Quantitative information on the significance, size and direction of allele frequency changes have previously been reported by Butrón et al. (2003). Some markers for which allelic frequencies significantly changed from expected under the

assumption of equal contribution of each inbred could be non neutral because they are located in locus involved in the responses of seeds or seedlings to *Fusarium* infection (*pathogenesis-related protein homolog2*), anoxia (*alcohol deshidrogenase2)*, oxidative (*catalase3*), heat and cold (*oxygen-evolving complex17*), and drought (*oleosin2*) stresses (http://www.maizegdb.org). The SSR markers located in the *pathogenesis-related protein homolog2* showed modified allelic frequencies compared to the expected frequencies under the assumption of equal contribution of each inbred line in EPS20r and EPS21r (Table 5). The frequencies of alleles at the marker *phi114,* located in the locus *oxygen-evolving complex17*, changed from the expected ones in the four synthetics.

Discussion

The lack of heterosis in crosses of EPS21r with the two Reid synthetics (EPS20c and EPS20r) could be attributed to the significant loss of diversity that was reported by Butrón et al. (2003) when the random method was used to develop the synthetic EPS21r. The superior agronomic performance of synthetics EPS20r and EPS21c along with their significant specific heterosis for yield might have caused EPS20r × EPS21c to yield more than the other crosses, except EPS20c × EPS21c. Therefore, from an agronomical point of view, the random method which is simpler and faster was beneficial when forming the synthetic with Reid-related inbreds, and was totally inappropriate when the base materials were unrelated inbreds. This finding is in partial agreement with predictions made by Butrón et al. (2003), based on the loss of genetic variability resulting from the use of the random method. Butron et al. (2003) hypothesized the superior performance of EPS21c compared to EPS21r, but no variability difference between EPS20c and EPS20r was found in that previous study.

Results of yield evaluations involving crosses between synthetics EPS20c and EPS20r and their parents were generally similar to the genetic relative distances between synthetics and their parental inbreds based on molecular data (Butrón et al. 2003). The close correspondence between genetic distances and yield performance supports the findings of previous studies that showed that among genotypes with similar pedigree background, there were high correlation coefficients between genetic diversity values estimated from field data and the genetic distances based on molecular markers (Williams and Hallauer 2000, Reif et al. 2003, García et al. 2004).

The different contributions of inbreds to each synthetic (EPS21c and EPS21r), detected at the molecular level (Butrón et al. 2003), was not always reflected on the yield

performance of crosses between synthetics and inbreds. The differences in the results of the two studies could be due to the fact that most markers were unrelated to genomic regions relevant to grain yield in this genetic background (Boppenmaier et al. 1992).

Nei's genetic distance between EPS20c and EPS20r was low and the yield of the cross EPS20c × EPS20r was about the same as the mid parental value while the cross between more genetically distinct synthetics, EPS21c × EPS21r, yielded more than the mean of their parents. This remarkable correspondence between genetic distances and midparent heterosis suggests that some markers could be indirectly related in yield performance. Therefore, the differences in allelic frequencies observed by Butrón et al. (2003) between synthetics obtained from the same materials by different methods could have been responsible for the differences in yield observed in the present study. This finding supported the idea of investigating, through data mining in the 'Maize Genetics and Genomics Database' (MaizeGDB), whether loci detected by Butrón et al. (2003) as showing allelic frequencies significantly different from expected (under the assumption of equal contribution of each inbred) are located on known genes that could be directly implicated in agronomic differences between synthtetics.

The causes of the departure from a model with equal contribution from each inbred to the synthetic could be two: (1) random allelic changes due to genetic drift and (2) directional selection. When using the random method, the contribution of directional selection to changes in allele frequencies from expected ones under equal contribution of each inbred could be important because the effective population sizes obtained with all loci were underestimated. On the contrary, selection did not have a big contribution to allelic changes when using the convergent cross method because the estimates of the effective population sizes were closed to the expected ones assuming that random drift was acting alone. However, the estimated population size computed with non neutral loci in EPS20c

differed from the expected one suggesting that selection could have some minor impact on frequency changes when using the convergent cross method in the Reid background, while no effect of selection was detected when using the same method in a more genetically diverse background.

The marker *phi114* exhibited allelic frequencies significantly different from expected in all synthetics with the allelic changes in the same direction when both methods were employed suggesting that natural selection for the locus *oxygen-evolving complex17*, where the maker is located, could have been acting when random and convergent methods were used. The locus *oxygen-evolving complex17* is involved in the response to cold stress. An important role in adaptation to abiotic stresses affecting water status, drought and cold, has also been suggested for the enzyme encoded by the locus *phosphoenolpyruvate carboxylase* (González et al. 2003) and the marker *phi065*, located on this locus, exhibited different allelic frequencies from expected in EPS20c. Natural selection for these loci was expected because the synthetics were developed at the Atlantic European area where cold is the most important stress (Malvar et al. 2005), but natural selection for cold tolerance did not seem to have contributed to agronomic differences between synthetics obtained from the same set of inbreds.

When using the convergent cross method, recombination of alleles from different inbreds occurs before random drift and/or selection could act. However, when using the random method, these factors could affect all allele frequencies of an inbred because they begin to act before any recombination of alleles occurs. Therefore, alleles whose frequencies had been significantly increased by the random method could correspond to markers linked to traits under selection, but could also be from inbreds that were favored by selection in the initial year (before alleles from different inbreds were recombined). Several SSRs that exhibited allelic frequencies different from expected in EPS20r and/or

EPS21r are not neutral because they are located in genes involved in responses to stresses. However, *phi083*, a marker located in the *pathogenesis-related protein homolog2*, was the only SSRs for which allelic frequencies were different from expected in both genetic backgrounds. All lines that increased their contributions to EPS20r and EPS21r, based on agronomic and previous molecular evaluations, supplied a fragment similar in size for the marker *phi083*; while CM151, A634, F473, and EP17, that showed reduced contribution to EPS20r or EPS21r, supplied SSR fragments for *phi083* with significant decrease in frequencies in those synthetics. Previously, *phi083* or a QTL linked to it was found to be involved in germination of aged seeds of the inbred P39 (Revilla personal communication). The inbred seed used to generate the synthetics was partially aged because it was not multiplied the year before inbred recombination. Therefore, we hypothesize that inbreds carrying unfavorable variation for the marker *phi083* could decrease their contribution to synthetics when using the random method because of reduced germination rate. Reedy et al. (1995) reported that differential survival in storage may result in changes in the genetic makeup of an accession by selection. Therefore, the differences in germination could favor the contribution of the B14-related inbreds to the synthetic EPS20r compared to WF9-related inbreds and, indirectly, could contribute to the increase of yield because B14 and their relatives are among the most promising elite inbreds (Lu and Bernardo 2001). However, natural selection in EPS21r against non-competitive inbred lines at germination would be responsible for the important reduction of variability that would affect yield performance because performance at germination is not always correlated to performance at later stages (Soldati et al. 1999).

In conclusion, results suggest that directional selection for germination was responsible for agronomic and genetic differences between synthetics obtained by alternative methods from the same set of inbreds. Selection for germination increased

the yield performance of the synthetic obtained from the Reid inbreds, but, in a more heterogeneous genetic background, natural selection against non-competitive inbred lines at germination would be responsible for an important reduction of variability that would reduce yield.

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Table 1. Maize inbred lines that were the base material for synthetic varieties EPS20 and EPS21, and their pedigrees

Synthetic variety	Inbred lines	Pedigree[a]	Group of germplasm[b]
EPS20	CM109	(V3 × B14) B14	Reid-B14
	CM139	(V3 × B14) B14	Reid-B14
	CM151	(Mt42 × WF9) WF9	Reid-WF9
	A634	(Mt42 × B14) $B14^3$	Reid-B14
	A639	A158 × B14	Reid-B14
	A652	A90 × WF9	Reid-WF9
	A664	(ND203 × A636) $A636^2$	Reid-B14
	W64A	WF9 × C.I. 187-2	Reid-WF9
EPS21	EP17	A1267	Spanish flint
	EP43	Parderrubias[c]	Spanish flint
	EP53	Laro[c]	Spanish flint
	PB60	Nostrano dell'Isola[c]	Italian flint
	PB130	Rojo Vinoso de Aragón[c]	Spanish flint
	F473	Doré de Gomer[c]	French flint
	CO125	Wisc. Exp. single cross	Corn Belt (USA)
	A509	A78 × A109	Corn Belt (USA)

[a] Pedigrees for the US inbreds are reported following Gerdes et al. (2003).

[b] B14 and WF9 are two inbred lines originated from the population "Reid" and were the origin of two groups of germplasm within the Reid material.

[c] Local European maize varieties.

Table 2. Mean grain yield (Mg ha^{-1}) of parental populations (on the diagonal) and diallel crosses (above the diagonal) among maize synthetics developed by two methods and midparent heterosis (below the diagonal).

	EPS20c	EPS20r	EPS21c	EPS21r
EPS20c[a]	5.4	6.0	7.1	6.2
EPS20r	-0.1	6.5	7.9	6.0
EPS21c	1.6*	1.9*	5.6	5.9
EPS21r	1.2	0.5	0.8	4.6

*Significantly different from zero at 0.05 probability level

[a] c was assigned to synthetics obtained by the convergent cross method and r to those obtained by the random method.

[b] The LSD was 1.3 for grain yield and 2.0 for midparent heterosis for yield.

Table 3. Mean comparisons among testcrosses of the maize synthetics EPS20c, EPS20r, EPS21c, and EPS21r to their parental inbred lines for yield (Mg ha^{-1}) evaluated for two years in Pontevedra (Spain).

Inbreds	EPS20c[a]	EPS20r	Inbreds	EPS21c	EPS21r
CM109	4.7	4.5	EP17	5.1	4.4
CM139	4.6	3.4	EP43	-	5.3
CM151	5.3	5.4	EP53	4.8	4.0
A634	4.8	5.8	PB60	5.2	5.2
A639	5.3	-	PB130	4.3	4.9
A652	5.4	6.3	F473	5.7	5.8
A664	5.8	4.6	CO125	4.8	4.0
W64A	5.1	6.0	A509	4.4	4.3
LSD	-	1.3	LSD	-	1.2

[a] c indicates synthetics obtained by the convergent cross method and r to those obtained by the random method.

Table 4. Estimates of the effective population size (N_e) and 95% confidence intervals[1] by the method of Waples (1989). The standardized variance in allele frequency change (F_c) was calculated following the method of Nei and Tajima (1981).

N_e	Loci	EPS20c	EPS20r	EPS21c	EPS21r
Estimated	All	77.18	25.35	69.25	15.37
		(31.59, 205.32)	(12.29, 46.96)	(33.23, 148.66)	(8.66, 24.87)
	Neutral[2]	188.75	57.73	162.09	59.75
		(54.08, ∞)	(22.69, 145.92)	(52.90, 1512.95)	(15.64, 235.43)
	Non neutral[3]	15.02	7.82	25.48	10.76
		(0.97, 63.68)	(1.23, 22.34)	(7.23, 67.12)	(5.42, 18.54)
Expected[4]		68.65	95.18	65.05	99.09

[1] 95% confidence intervals are within brackets.

[2] Loci that exhibited allelic frequencies non significantly different from the expected ratios.

[3] Loci that exhibited allelic frequencies significantly different from the expected ratios.

[4] The expected N_e was the harmonic mean of the individuals crossed in each segregating generation: 40, 104, and 110 for EPS20c; 76, 106, and 112 for EPS20r; 36, 109, and 109 for EPS21c; and 78, 122, and 108 for EPS21r.

Table 5. SSR markers for which allelic frequency were significantly different from expected ratios if an equal contribution of each parental inbred line is assumed, indicating the maize inbred lines that reduced and increased their contributions to the synthetic (original data in Butrón et al. 2003) and the gene where the SSR marker is located.

Synthetic	SSR marker	Inbred lines (reduced)	Inbred lines (increased)	Gene
EPS20r	*phi083*	CM151, A634	CM109, CM139, A639, A664	*pathogenesis-related protein homolog2*
EPS20c and EPS20r	*phi114*	CM109, CM139 CM151, A652, W64A	A634, A639, A664	*oxygen-evolving complex17*
EPS20r	*bnlg240*	CM151, A652	W64A	unknown
EPS20c	*phi065*	CM109, CM139, W64A	A634, A639, A652 A664	*phosphoenolpyruvate carboxylase1*
EPS20r	*phi050*	CM151	CM109, CM139, A634, A639, A652, A664, W64A	unknown
EPS21r	*phi083*	EP17, F473	CO125, A509	*pathogenesis-related protein homolog2*

EPS21c	*phi036*	PB60, CO125, EP53	EP43	unknown
EPS21r		EP17, F473, EP53	EP43, PB60, CO125	
		PB130, A509		
EPS21c	*bnlg197*	EP17, PB60, A509, PB130	EP53, CO125, EP43, F473	unknown
EPS21r		EP17, PB60, EP43, F473	EP53, CO125, A509, PB130	
EPS21r	*phi021*	PB60	CO125	*alcohol dehidrogenase2*
EPS21r	*phi076*	EP17, EP43, F473, A509	EP53, PB60, PB130, CO125	*catalase3*
EPS21r	*phi113*	EP17, EP43, EP53, PB60	A509	*oleosin2*
		PB130, F473, CO125		
EPS21r	*phi075*	EP17, F473	EP43, PB130, CO125	*ferrodixin1*
EPS21c	*phi114*	EP17, EP43, PB60, A509	PB130, F473	*oxygen-evolving complex17*
EPS21r		EP17, EP43, PB60, A509	EP53, PB130, F473, CO125	
EPS21r	*phi101*	EP17, PB60	CO125	unknown